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Predicting the behaviour of *Yersinia enterocolitica* and *Listeria monocytogenes* in Italian style fresh sausages under drying period

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Abstract

Italian style fresh sausage is a traditional pork product that is frequently consumed raw, usually after drying. So far, the prediction of the behaviour of microorganisms in varying environments has been carried out through separate growth or death models. The aim of this study is to show how to combine these, in order to describe the kinetics of *Yersinia enterocolitica* and *Listeria monocytogenes* in sausage during the drying period.

During this time, the storage temperature and the pH are fairly constant, while the water activity decreases, being the primary cause of shifting the organisms' kinetics from growth to inactivation. The water activity takes up values from growth (R_G), uncertainty (R_0) and death (R_D) regions. In the uncertainty region, neither growth nor death is predicted. Among the native flora, lactic acid bacteria may have significant effect on these kinetics.

Here we show how to generate predictions for such scenarios, from data that are generated in constant environments. Thus the predictions in our dynamic environments are extrapolations and could be problematic if the environmental fluctuation is extreme. Our approach can be used to predict bacterial growth/death kinetics under temporal variation of storage environments, which is vital when assessing the safety of fresh sausage. We envisage similar applications to other RTE meat products, too.

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1. Introduction

Italian style fresh sausage is a traditional pork product. It can be consumed cooked or raw. The consumption of raw sausage is most frequent in regions of Central Italy, usually after a variable drying period. According to regulation CE 853/2004¹ the process should be considered to be a meat preparation, but since the drying period can change the

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physical characteristics of the meat, the result could be a “salami-style” meat product. In Italy, sausages are usually self-checked or “intended to be cooked” products. In some Italian regions, as high as 5% of them are eaten raw².

Codex Alimentarius recommends to move food control activities from a hazard-based and final testing to a more risk-based management approach^{3,4}. This has made statistical and mathematical tools specifically designed to predict microbial behaviour more and more necessary. The use of predictive models is also recommended by EC regulation 2073/2005⁵, currently in effect in the EU. This regulation makes it possible for food business operators to use “predictive mathematical modelling established for the food in question, using critical growth or survival factors for the micro-organisms of concern in the product”. Though predictive models cannot completely replace lab testing or the judgement of an expert food microbiologist, but they aid informed decisions on food safety issues⁶.

So far, the prediction of the behaviour of microorganisms in varying environments has been carried out through separate growth or death models. The aim of this paper is to show how to develop models to predict the kinetics of these pathogens in dynamic environments shifting from growth to inactivation regions. The parameters of existing predictive models for *Y. enterocolitica* and *L. monocytogenes*⁷ will be used for demonstration.

2. Materials and methods

2.1 Primary models of growth and death

For constant, growth-supporting environments, the log counts data can be fitted by the explicit (4-parameter sigmoid curve) version of the model of Baranyi and Roberts⁸:

$$y(t) = y_0 + \mu A(t) - \text{Ln} \left(1 + \frac{e^{\mu A(t)} - 1}{e^{y_{\max} - y_0}} \right)$$

$A(t)$ being

$$A(t) = t - \lambda + \frac{\text{Ln}(1 - e^{-\mu t} + e^{-\mu(t-\lambda)})}{\mu}$$

where

- $y(t)$ describes the temporal variation of the natural logarithm of the bacterial concentration, the time being denoted by t ;
- y_0 is the natural logarithm of the initial bacterial concentration (i.e. at $t=0$);
- y_{\max} is the natural logarithm of the maximum population density the given environment and resources are able to carry;
- μ is the maximum specific growth rate (the maximum of the dy/dt rate), characteristic of the growth environment.
- λ is a lag parameter (the time point where the actual specific rate is $\mu/2$).

This is an algebraic solution of the more general, dynamic model of Baranyi and Roberts⁸:

$$\begin{cases} \frac{dy}{dt} = \frac{1}{1+q} \mu (1 - e^{y_{\max} - y}) \\ \frac{dq}{dt} = vq \end{cases} \quad (1)$$

where $q(t)$ is a certain quantification of the actual physiological state of the cells that continuously improving according to linear kinetics, immediately after the inoculation, and the specific rate depends on $\ln(q)$ according to a

Michaelis-Menten inhibition function. The specific “improvement-rate” of q is assumed (empirically) to be equal the maximum specific rate of the cells: $\mu=v$.

The lag time does not appear directly in the generic, differential-equation form of the model. The lag is in fact a derived parameter determined partly by the actual environment, partly by the initial physiological state of the cells, quantified here by q_0 . As the cells gradually adjust to their new environment, $\ln q(t)$ increases linearly. Baranyi and Roberts⁸ showed that if the dynamic version is used for constant environment then, with the reparameterisation $\alpha_0=1/(1+q_0)$, the lag in the static environment will be

$$\lambda = \frac{h_0}{\mu} = \frac{-\ln \alpha_0}{\mu}$$

Any of the q_0 , α_0 , h_0 quantifications can be considered an initial value (apart from y_0) for equation (1), they all measure the history effect, though their biological interpretations are different. The formula expresses the expectation that the lag time depends on both the current environment (via μ) and the history of the cells (via h_0).

The above model can be applied to the R_G growth region of the environmental factors. In the R_0 and R_D death regions, simple linear kinetics can be used; i.e. the temporal variation of the natural logarithm of the cell concentration is modelled by

$$\frac{dy}{dt} = 0 \quad (\text{in } R_0) \quad \text{and} \quad \frac{dy}{dt} = \mu_d \quad (\text{in } R_D) \quad (2)$$

2.2 Secondary models for growth and inactivation.

The effects of the environment on the growth and death rate of *L. monocytogenes* and *Y. enterocolitica* are modelled in ComBase Predictor by a simple quadratic response surface, where the environment is characterized by three variables: temperature (T), pH and b_w . The latter variable is a rescaled version of the water activity⁹:

$$b_w = \sqrt{1 - a_w}$$

That is:

$$\ln \mu = a_0 + a_1 T + a_2 pH + a_3 b_w + a_4 T \cdot pH + a_5 T \cdot b_w + a_6 pH \cdot b_w + a_7 T^2 + a_8 pH^2 + a_9 b_w^2 \quad (3)$$

Note that these coefficients are based on experiments where the pathogens were grown in culture medium, under constant environments.

In order to predict the death rate (μ_d), similar polynomial model can be used based on ComBase Predictor. The coefficients ($b_0 \dots b_9$) are now:

$$\ln \mu_d = b_0 + b_1 T + b_2 pH + b_3 b_w + b_4 T \cdot pH + b_5 T \cdot b_w + b_6 pH \cdot b_w + b_7 T^2 + b_8 pH^2 + b_9 b_w^2 \quad (4)$$

2.3 Food and storage environment.

So far we have the necessary models and their parameter values to make predictions for experiments in culture medium as the parameters were derived in such circumstances. For practical predictions in food storage environments, three additions are necessary:

1. An estimation of the initial values, which is determined by the history of the cells;
2. An assessment of the difference between the culture medium and the food matrix in question; and
3. An assessment whether the dynamic environment is an uncertain extrapolation from the static environment used in the experiments generating the data on which the parameter estimation is based.

For a solution these considerations should be made:

1A) The y_0 initial contamination level can be estimated by a risk assessment procedure, based on the processing and storage history of the food. This is an input for the simulation and out of the scope of the current study. It is common in predictive software packages that the $y_0=0$ initial value is used (1 cell/g bacterial concentration) and a threshold is drawn like half or one log unit increase, above which the safety is compromised.

1B) The initial physiological state, or the equally applicable h_0 “work to be done” during the cells’ adaptation to the sausage environment after contamination needs some challenge tests. This value is fairly constant (between 2 and 3) in culture medium experiments, in static and growth-supporting environments but can be very different in food as it expresses a bridge between pre- and post-contamination environment.

2) The effect of the difference between the culture medium and the sausage appears in the well-analysed question of *bias*¹⁰. In the main, this is a difference between the $\text{Ln}(\text{rate})$ values of the two systems, which can be transformed to the *bias factor* between them, preferred by Ross¹¹. The corner stone of this approach is the reliability of the assumption that the bias (or bias factor) is constant in the studied region of the environmental factors. By all means the challenge tests, needed to establish h_0 , also need to be used to estimate the bias.

Among the natural microflora of fresh sausage, lactic acid bacteria (LAB) affect the kinetics of *Listeria monocytogenes* and *Yersinia enterocolitica* growth the most. This effect on μ_{\max} can be also included in the overall bias.

3) The basic assumption behind using the equations (1) and (2) for simulation is that the specific growth/death rate of the cell population instantaneously takes up the value corresponding to the one predicted under static conditions. This assumption could be wrong in extremely fluctuating environments. However, in our situation the water activity is decreasing slowly and monotonously therefore we do not calculate with possible complications. It is important, however, the estimation of the water activity profile, which demands water activity measurements during the challenge tests.

2.4 Numerical procedures

In order to predict bacterial variation throughout the growth/death regions, threshold value of the water activity was used to define the boundaries of these regions. These threshold values can be obtained from literature data.

Depending on the region of the environmental factors, equations (3) and (4) define the rates μ or μ_d that can be used to simulate the growth of the pathogens in question by solving the system defined by (1) and (2). Normally it is a futile effort to try to find an algebraic solution but even a simple Excel spreadsheet can be programmed using for example Euler’s method for simulation.

3. Results and discussion

The expected behaviour of the growth/death rate of pathogens during the drying period of the sausage is demonstrated in Fig.1. The transition of the rate between the regions is not smooth (non-differentiable), but this hardly decreases the accuracy of the prediction.

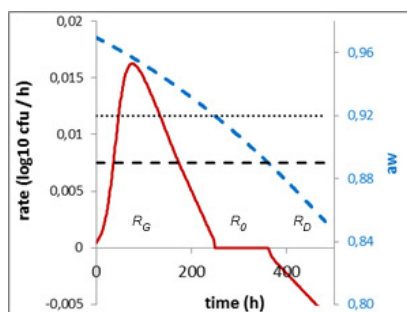


Fig.1. Water activity (a_w , thick broken line) decreases during 3 weeks of drying period. At high a_w , the organism grows, and a predictive model will provide growth rate at this R_G region. At low a_w , the rate is negative (R_D region). Between the growth and death region, the kinetics is so uncertain that neither growth nor death is predicted (R_0). The boundary of regions (thin broken and dotted lines) are from literature studies.

4. Conclusion

Different growth and inactivation models are used in food industry to evaluate shelf-life and comply with microbial criteria. Microbial kinetics, in many products with long shelf-life and dynamic internal environment, could result in both growth and inactivation, making difficult to estimate the bacterial concentration at the time of consumption by means of commonly available predictive software tools. Our approach, built on the storage environments of traditional Italian meat products, where the water activity gradually changes during a drying period, is designed to overcome these difficulties. An uncertainty region (R_0) has been inserted in the growth and death regions on the basis of well-defined boundary levels of water activity. Neither growth nor death was predicted in this region, being a “transition zone” between the other two.

The main question of applying predictive models to practical scenarios is what extent of extrapolations can be made without compromising the accuracy of the models. Here we pointed out that though arbitrarily changing, such as extremely fluctuating environment could corrupt the model based on measurements in static environments, gradual “smooth” change, such as the decrease of water activity during drying, should not cause problems, inasmuch the dynamic version of the predictive model is used (for which the static environment is just a special case). Other extrapolating steps, like inferring kinetics from culture medium to food matrix by a single bias factor, of estimating and summarizing the effect of history by a single initial physiological state parameter, need to be tested by a few challenge tests. It would be unwise to extrapolate predictions based on culture medium and static environments without any tests.

Our approach can be used to predict and visualize bacterial kinetics under temporal variation of environments, which is vital when assessing the safety of many products, such as fresh sausage.

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